

Claims

1. A method of producing a genetically modified mammalian cell, said method comprising the steps of:

(a) inserting into one or more mammalian cells an artificial chromosome comprising a cassette which includes a first region of homology having substantial sequence identity to a first region of an endogenous chromosome of said mammalian cell(s), a selectable marker, and a second region of homology having substantial sequence identity to a second region of said endogenous chromosome under conditions that result in homologous recombination between said artificial chromosome and said endogenous chromosome and integration of said cassette into said endogenous chromosome of one or more mammalian cells; and

(b) selecting a mammalian cell in which said homologous recombination occurs, thereby selecting a genetically modified mammalian cell.

2. The method of claim 1, wherein said artificial chromosome comprising said cassette is produced by a method comprising the steps of:

(a) culturing a host cell that has (i) a linear DNA molecule comprising said cassette and (ii) an artificial chromosome comprising nucleic acid that is substantially identical to said first and second regions of homology under conditions that result in homologous recombination between said linear DNA molecule and said artificial chromosome, thereby generating said artificial chromosome comprising said cassette.

3. The method of claim 2, wherein said linear DNA molecule is introduced into said host cell by transformation.

4. The method of claim 2, wherein said linear DNA molecule is introduced into said host cell by insertion of a circular vector comprising the sequence of said linear DNA molecule into said cell and cleavage of said vector to generate said linear DNA molecule inside said host cell.
5. The method of claim 1, wherein said first and second regions of said endogenous chromosome are contiguous.
6. The method of claim 5, wherein said first and second regions of said endogenous chromosome are part of the same exon or the same promoter.
7. The method of claim 1, wherein said first and second regions of said endogenous chromosome are not contiguous.
8. The method of claim 7, wherein said first and second regions of said endogenous chromosome are part of different exons.
9. The method of claim 1, wherein the integration of said cassette into the genome of said mammalian cell reduces the activity of the protein encoded by a nucleic acid of interest.
10. The method of claim 1, wherein the amount of functional protein encoded by said nucleic acid of interest decreases by at least 25%.
11. The method of claim 1, wherein said cassette comprises a reporter gene, and wherein said cassette is integrated into the genome of said mammalian cell such that said reporter gene is operably linked to an endogenous promoter of interest, thereby generating a genetically modified mammalian cell that expresses said reporter gene under the control of said promoter.

12. The method of claim 1, wherein said cassette comprises a nucleic acid encoding a detectable protein, and wherein said cassette is integrated into the genome of said mammalian cell such that said nucleic acid is operably linked to an endogenous nucleic acid encoding a protein of interest, thereby generating a genetically modified mammalian cell that expresses a fusion protein comprising said detectable protein and protein of interest or fragment thereof.

13. The method of claim 1, further comprising repeating steps (a) and (b), thereby generating a genetically modified mammalian cell with two or more mutations.

14. The method of claim 13, wherein each cassette comprises a recombinase signal sequence, thereby generating a genetically modified mammalian cell with two recombinase signal sequences.

15. The method of claim 14, wherein recombination occurs between said recombinase signal sequences in said mammalian cell.

16. The method of claim 15, wherein said recombinase signal sequences are in the same endogenous chromosomes of said mammalian cell, and wherein recombination between said recombinase signal sequences results in elimination of the DNA between said recombinase signal sequences.

17. The method of claim 15, wherein said recombinase signal sequences are in different endogenous chromosomes of said mammalian cell, and wherein recombination between said recombinase signal sequences results in chromosomal translocation between said recombinase signal sequences.

18. The method of claim 1, wherein said mammalian cell is an embryonic stem cell.

19. The method of claim 1, wherein said mammalian cell is a somatic cell.

20. A method of producing a genetically modified non-human mammal, said method comprising inserting a mammalian cell produced by the method of claim 1 into a non-human embryo under conditions that allow said embryo to develop into a fetus.

21. A method of producing a genetically modified non-human mammal, said method comprising the steps of:

(a) inserting a mammalian cell produced by the method of claim 1 or a nucleus from said cell into an oocyte; and

(b) transferring said oocyte or an embryo formed from said oocyte into the uterus of a host mammal under conditions that allow said oocyte or said embryo to develop into a fetus.

22. The method of claim 20 or 21, wherein said fetus develops into a live offspring.

23. The method of claim 22, further comprising mating two of said offspring to generate a mammal with a homozygous mutation.

24. The method of claim 22, further comprising mating two of said offspring to generate a mammal with a mutation in two or more genes.

25. The method of claim 20 or 21, wherein said cell comprises two recombinase signal sequences and recombination occurs between said recombinase signal sequences in cells of a predetermined cell type of said fetus or a live offspring formed from said fetus.

26. The method of claim 20 or 21, wherein said cell is an embryonic stem cell.

27. The method of claim 20 or 21, wherein said cell is a somatic cell.

28. The method of claim 20 or 21, wherein said mammal is a murine, bovine, ovine, porcine, or caprine.

29. The method of claim 28, wherein said mammal is a murine.

30. A screening method for determining whether a candidate compound modulates the expression of nucleic acid of interest, said method comprising the steps:

(a) administering a candidate compound to a mammal that has a genetic modification in a nucleic acid of interest or in a promoter operably linked to a nucleic acid of interest and that is produced by the method of claim 20 or 21; and

(b) measuring expression of a nucleic acid of interest, whereby said candidate compound is determined to modulate expression of said nucleic acid if said candidate compound causes a change in expression of said nucleic acid.

31. The method of claim 30, wherein step (b) comprises measuring the expression of an mRNA corresponding to said nucleic acid.

32. The method of claim 30, wherein step (b) comprises measuring the expression of a protein encoded by said nucleic acid.

33. The method of claim 30, wherein said mammal is genetically modified to express a reporter gene operably linked to a promoter of interest.

34. The method of claim 30, wherein said mammal is genetically modified to express a fusion protein comprising a detectable protein and a protein of interest or a fragment thereof.

35. A screening method for determining whether a candidate compound is useful for the treatment, stabilization, or prevention of a disease, disorder, or condition, said method comprising the steps:

(a) administering a candidate compound to a mammal produced by the method of claim 20 or 21; and

(b) measuring one or more symptoms associated with a disease, disorder, or condition, whereby said candidate compound is determined to be useful for the treatment, stabilization, or prevention of said disease, disorder, or condition, if said candidate compound reduces, stabilizes, or prevents said symptom.

36. The method of claim 35, wherein said mammal has a chromosomal deletion or translocation associated with cancer.

37. A method for determining whether a nucleic acid is associated with a disease, disorder, or condition, said method comprising measuring one or more symptoms associated with a disease, disorder, or condition in a mammal that has a mutation in a nucleic acid of interest and that is produced by the method of claim 20 or 21, whereby said nucleic acid is determined to be associated with said disease, disorder, or condition if said symptom differs between said mammal and a control mammal without said mutation.

38. A method of treating, stabilizing, or preventing a disease, disorder, or condition in a mammal, said method comprising administering one or more cells produced by the method of claim 1 to a mammal in an amount sufficient to treat, stabilize, or prevent said disease, disorder, or condition.

39. The method of claim 38, wherein said mammal is a human.